

Immunovirological outcomes and resistance patterns at 4 years of antiretroviral therapy use in HIV-infected patients in Cambodia

Mar Pujades-Rodríguez¹, Birgit Schramm¹, Leakena Som², Eric Nerrienet³, Prak Narom⁴, Ngeth Chanchhaya⁴, Laurent Ferradini² and Suna Balkan⁵

¹ *Epicentre, Paris, France*

² *Médecins Sans Frontières, Phnom Penh, Cambodia*

³ *HIV/Hepatitis Laboratory, Pasteur Institute, Phnom Penh, Cambodia*

⁴ *Khmero-Sovietic Friendship Hospital, Phnom Penh, Cambodia*

⁵ *Médecins Sans Frontières, Paris, France*

Summary

OBJECTIVES To report immunovirological outcomes and resistance patterns in adults treated with triple combination antiretroviral therapy (cART) for 4 years in an HIV programme of Phnom Penh, Cambodia.

METHODS It is a longitudinal study and cross-sectional evaluation of adults receiving cART for 4 years. CD4 cell counts and HIV-1 RNA were quantified, and resistance patterns were determined. Drug-related toxicity was assessed by clinicians and through laboratory testing.

RESULTS After 4 years of cART start, the cumulative probability of retention in care was 0.80 and survival among patients not lost to follow-up was 0.85. A total of 349 patients (98% of eligible) participated in the cross-sectional evaluation. Ninety per cent were receiving first-line therapy, 29% stavudine- and 58% zidovudine-containing regimens (compared with 94% and 3% at cART initiation). Ninety-three per cent of patients were clinically asymptomatic, and severe lipodystrophy and dyslipidemia were diagnosed in 7.2% and 4.0%, respectively. Good treatment adherence was reported by 83% of patients. Median CD4 T-cell count was 410 cells/ μ l [IQR 290–511], and 90% of patients had >200 cells/ μ l. Only 15 (4%) patients had detectable HIV viral load (eight had <200 CD4 cells/ μ l), five had thymidine analogue mutations, and nine were resistant to two drug classes. In an intention-to-treat analysis, 26.1% (95% CI 22.0–30.5) of patients had failed first-line therapy.

CONCLUSIONS In this Cambodian cohort of adults who started cART at an advanced stage of HIV disease, we observed good clinical and immunovirological outcomes and self-reported treatment adherence at 4 years of therapy.

keywords HIV, programme evaluation, treatment failure, drug resistance, developing countries

Introduction

The provision and scale-up of HIV/AIDS treatment in resource-limited countries has been possible through a public health approach consisting of a limited biological follow-up, strengthening of adherence and use of generic, fixed-dose combinations (FDC) of antiretroviral (ARV) drugs. Recent evaluations of clinico-immunological outcomes of patients using this approach have shown the feasibility and effectiveness of triple combination ARV therapy (cART) provided to patients treated in these settings (Walsh *et al.* 2002; Orrell *et al.* 2003; World Health Organization, 2006; Ferradini *et al.* 2007). Regular

evaluations of immunovirological response, tolerability, and patient adherence to therapy in clinical practice are scarce but necessary to determine the long-term effectiveness of programmes. They are also essential to optimize strategies of care that maximize the duration of first-line cART.

In 2001, Médecins Sans Frontières (MSF), in collaboration with the Cambodian Ministry of Health, started providing free cART to HIV-infected patients at the Khmer Soviet Friendship Hospital of Phnom Penh, Cambodia. We reported previously the favourable immunovirological results of adults treated with cART for 2 years in this programme (Ferradini *et al.* 2007). The objective of this

study was to describe immunovirological treatment outcomes and ARV-related adverse events diagnosed in patients receiving cART for 4 years.

Methods

HIV programme of Phnom Penh

HIV-infected patients treated in the HIV programme of Phnom Penh attend regular clinic visits by physicians and receive intensive counselling and education support by experienced counsellors before (at least two pre-ART individual sessions) and after cART start (every month during the first 4 months and every 6 months thereafter). Patient immunological monitoring is performed every 6 months, but routine virological testing is not available.

First-line therapy provided in the programme is a fixed-dose combination of two nucleoside reverse transcriptase drugs (NRTI), generally stavudine (d4T) and lamivudine (3TC) and one non-NRTI (NNRTI), nevirapine (NVP) or efavirenz (EFV). Alternative first-line regimens are available in case of occurrence of side effects [e.g. d4T could be replaced by zidovudine (AZT), and AZT could be replaced by tenofovir (TDF)]. Second-line therapy containing boosted lopinavir (LPV/r) and didanosine (ddI) and one or two additional NRTI drugs was provided, when treatment failure was diagnosed (WHO 2003 criteria).

Tuberculosis diagnosis is based on the results of microscopic examination of body specimens (e.g. sputum, pleural effusion, cerebrospinal fluid) for acid-fast bacilli, and/or radiological findings suggestive of active pulmonary tuberculosis performed in patients with clinical suspicion of the disease. To differentiate between tuberculosis and non-tuberculosis mycobacteria, since 2006, culture is systematically performed for suspected tuberculosis cases with negative direct examination results.

Study population and procedures

Adults aged 18 years or older, excluding pregnant women, who started treatment between October 2002 and June 2003 (48 ± 2 months before the study inclusion period) in the MSF-supported HIV programme in Phnom Penh and still receiving treatment were invited to participate in a cross-sectional survey. After obtaining written informed consent, information on patients' age, sex, body mass index (BMI) and treatment history was collected. Additionally, data on the presence of ARV-related adverse events in the previous 30 days were collected by physicians using standardized questionnaires. Patients were asked whether they experienced specific symptoms from a pre-defined list and whether these

interfered with normal activity. Adverse events were graded according to WHO guidelines (World Health Organization, 2006).

During the study visit, non-medical counsellors assessed compliance with cART using a 10-point visual analogue scale (VAS) to assess self-reported adherence over the previous month (Walsh *et al.* 2002). Patients were asked to rate themselves concerning the amount of ARV medication taken during the previous month (0 = no medication taken; 10 = all medication taken). They were classified as fully adherent if they had a VAS of 10 (all pills taken as prescribed) and poorly adherent if they had a VAS <10.

Laboratory testing

Blood specimens were collected from fasting patients, and concentrations of haemoglobin, creatinine, transaminases, alkaline phosphatases, glucose, triglycerides and both total and low high-density lipoprotein (HDL) cholesterol were measured. The number of blood platelets and neutrophils was also determined. Plasma HIV-1 RNA viral load was quantified with real-time polymerase chain reaction (G2 generic HIV viral load ANRS kit, Biocentric, Bando, France; limit of detection, 250 copies/ml) at the Pasteur Institute of Phnom Penh. HIV genotyping was performed for patients with virological failure (viral load >1000 copies/ml), reverse transcriptase genotyping was performed for all patients, and protease genotyping additionally for those receiving second-line therapy. To do this, HIV RNA was extracted from plasma using standard methods developed by the Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS) French Resistance Study Group (<http://www.hivfrenchresistance.org/tab2007.html>). Phylogenetic analysis was performed in both reverse transcriptase (RT) and protease (PR) genes. PCR amplified fragments were sent to the MacroGen Company (MacroGen Inc., Seoul, Korea). Chromatograms sent back electronically to the Pasteur Institute of Cambodia were verified, analysed and interpreted. Clustal X 1.81 software was used for alignment with subtype reference sequences set from the Los Alamos HIV sequence Database (http://hivweb.lanl.gov/content/HIVdb/SUBTYPE_REF/align.html) as well as for phylogenetic analysis using a nucleotide-distance matrix and the bootstrap neighbour-joining method. The resistant mutations were defined according to the WHO HIV Drug resistance database (<http://hivdb.stanford.edu/pages/WHOResistanceList.html>), and the drug susceptibility was predicted according to the ANRS algorithm (<http://www.hivfrenchresistance.org/>). CD4 T-cell counts were measured by flow cytometry (FACScount; Becton Dickinson, Franklin Lakes, NJ, USA).

Data management and analysis

Data were double-entered in EpiData 3.1 (Odense, Denmark), and analyses performed in Stata 9.0 (Stata Corp., College Station, TX, USA). Patient information was right-censored at death, last visit for lost to follow-up, transfer to another programme or at 48 months for other patients. Patients lost to follow-up were those who had missed their last clinical appointment by 2 months or more. Kaplan–Meier methods were applied to estimate probabilities of survival and retention in care. CD4 cell counts were categorized as follows: <50, 50–199, 200–499, ≥ 500 cells/ μl . Chemistry laboratory values were classified into severity grades according to recommended WHO grading schemes (World Health Organization, 2006). Absolute and CD4 cell gains by initial CD4 cell level were compared with Wilcoxon rank-sum tests. Differences in adherence, toxicity and virological failure status in men and women, and differences in adherence in patients with and without detectable viral load were tested with chi-square or Fisher's exact tests, as appropriate. Coefficient correlations between adherence and virological failure status were calculated. Finally, an intention-to-treat analysis was performed to estimate the rate of virological failure to first-line therapy within the subgroup of patients who survived 3 months of cART, assuming that all deaths and lost to follow-up patients had experienced treatment failure.

The study was approved by the National Ethics Committee of Cambodia and the Consultative Committee for People involved in Biochemical Research (CPRB) of Saint Germain-en-Laye, Paris, France.

Results

A total of 467 adults had started cART in the MSF-supported HIV/AIDS programme of Phnom Penh between 24 October 2002 and 23 June 2003. Since the start of therapy, 63 (14%) patients had died, 29 (6%) had been lost to follow-up, and 14 (3%) had been transferred to other health facilities (Figure 1). The cumulative probability of retention in care at 4 years of cART was 0.80 (95% CI 0.76–0.84), and cumulative survival among those not lost to follow-up was 0.85 (95% CI 0.82–0.88). At the time of the study, four women were pregnant, and eight patients attended the clinic before 46 or after 50 months of cART start (one of the eight had detectable viral load) and were therefore not eligible for inclusion in the cross-sectional survey. A total of 349 patients (98% of those eligible) participated in the evaluation.

At cART start, patient median age was 34 years, 94% were ARV naïve, and 24% had been diagnosed with tuberculosis (Table 1). Ninety-seven per cent of patients received d4T-containing therapy, and the majority started treatment at an advanced stage of HIV disease (59% in stage 4 and 70% had a CD4 count <50 cells/ μl).

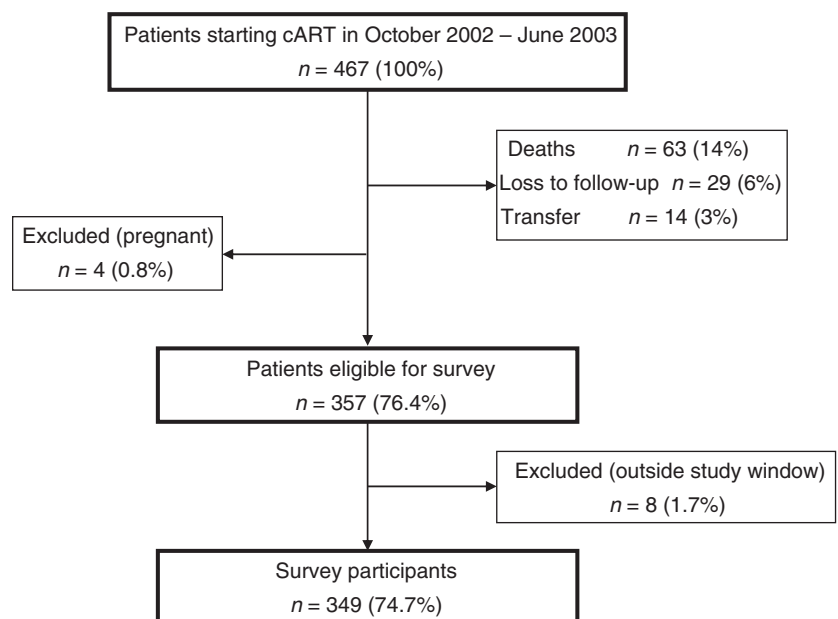


Figure 1 Study flowchart, Phnom Penh, Cambodia.

Table 1 Patient characteristics at antiretroviral therapy start and at 4 years of therapy use, Phnom Penh, Cambodia

	Study participants (N = 349)		
Characteristics at cART start			
Men (%)	137 (60.5)		
Median age, years [IQR]	34 [31–39]		
ART naive (%)	328 (94.0)		
d4T-3TC-EFV regimen (%)	287 (82.7)		
WHO stage 4 (%)	207 (59.3)		
Body mass index <18.5, kg/m ² (%)	143 (43.9)		
Median CD4 T-cell count, cells/ μ l ³ [IQR]	16 [4–71], n = 324		
Characteristics at survey			
Non-cumulative WHO stage at survey (%)			
Asymptomatic	326 (93.4)		
Stage 3 or 4	8 (2.2)		
Median body mass index, kg/m ² [IQR]	20 [19–23]		
Second-line LPV-based therapy (%)	36 (10.3)		
Severe reported clinical ARV toxicity (%)	Grade 3	Grade 4	
Digestive disorders	1 (0.3)	–	
Neurological disorders	1 (0.3)	–	
Dermatological disorders	1 (0.3)	–	
Lipodystrophy	25 (7.2)	–	
Severe laboratory-based ARV toxicity (%)	Grade 3	Grade 4	
Haematological disorders	2 (0.6)	2 (0.6)	
High ALT or AST	4 (1.2)	–	
Hypertriglyceridemia	14 (4.0)	10 (2.9)	
High total cholesterol	2 (0.6)	–	
Reported adherence over the last month (%) [*]			
Fully adherent	289 (83.3)		
Poorly adherent	12 (3.4)		
CD4 cell count, cells/ μ l [IQR]			
Median	410 [290–511]		
Median gain since ART start	+354 [262–474]		
HIV-1 RNA <250 copies/ml (%)	334 (95.7)		

3TC, lamivudine; ALT, alanine transferase; AST, aspartate transaminase; ARV, antiretroviral; cART, combined antiretroviral therapy; d4T, stavudine; IQR, interquartile range; LPV, lopinavir; NVP, nevirapine. Limits for toxicity of grade 3 were the following: for haematological disorders: anaemia 6.5–6.9 g/dl, hyperleucocytosis 1000–1500/mm³, neutrophilia 500–749/mm³, and thrombocytosis 20 000–49 999/mm³; for AST and ALT >5–10 × upper normal range limit; for total hypercholesterolaemia 10.34–12.92 mmol/l.

^{*}Self-reported adherence during the past month using a 10-point visual analogue scale was missing for one patient.

Clinico-immunological outcomes

At 4 years of cART, 93% of patients were asymptomatic (WHO classification), and 267 (76%) had BMI of

≥18.5 kg/m² (1% had BMI <16). Ninety per cent of patients were receiving first-line therapy, 29% d4T- and 58% AZT-containing regimens, and 36 (10%) had been switched to LPV/r-containing second-line therapy because of treatment failure. Overall, 29.5% of individuals had reached CD4 T-cell values ≥500 cells/ μ l, 63.2% of these had initial CD4 counts <50 and 35.8% 50–200. Nevertheless, 35 (10.0%) patients had CD4 T-cell counts <200 cells/ μ l, and 3 (0.9%) <100 cells/ μ l. Median CD4 increases were 101 [IQR 64–141; n = 309] at 6 months, 151 [96–213; n = 312] at 1 year, 245 (159–336; n = 308) at 2 years and 356 cells/ μ l [263–474; n = 324] at 4 years of cART. Median CD4 gains were higher in patients with lower initial CD4 cell counts, but higher median values were observed in individuals with higher initial CD4 measurements ($P = 0.002$ for both comparisons; Figure 2). Patients with <50 CD4 cell counts/ μ l at ART start had a median count of 392 cells/ μ l [IQR 280–506] and median gain of 380 [IQR 271–495], and those with ≥100 cells/ μ l had median 473 [411–570] and 321 [IQR 263–427], respectively. A total of 109 (31.2%) patients reached their highest CD4 T-cell values after 4 years of cART use.

ARV-related adverse events and laboratory abnormalities

Most individuals (80%) reported clinical adverse events, namely morphological disorders (63%), asthenia (32%), peripheral neuropathy (25%) and digestive symptoms (21%). These were mostly of mild to moderate severity. Intolerance of grade 3 was diagnosed in 31 (9%) patients; no grade 4 clinical toxicity was recorded (Table 1). One patient with severe neuropathy was receiving TDF-containing therapy but had previously been treated with d4T for 17 months and with AZT for 23 months. Grade 3 morphological disorders were diagnosed in 25 patients who had taken d4T for a median of 29 months; six of them were still receiving d4T-containing therapy at the time of the survey.

Eighty-five per cent had at least one abnormal laboratory measurement, specifically dyslipidemia (73%) and increased liver enzymes (27%). Only 1% had anaemia. Of the 26 patients with mild to moderate high creatinine levels (16% of patients on second line and 6% of those on first line), only three were receiving TDF-containing therapy for a median of 14 months; six were receiving boosted LPV-containing regimens; and 17 a combination of 3TC with d4T or AZT, and EFV or NVP. Grade 3 or 4 laboratory abnormalities were observed in 36 (10%) patients. Four of the 24 patients with severe hypertriglyceridemia were receiving second-line regimens (11% compared to 6% of individuals on first line), and one of the three patients with

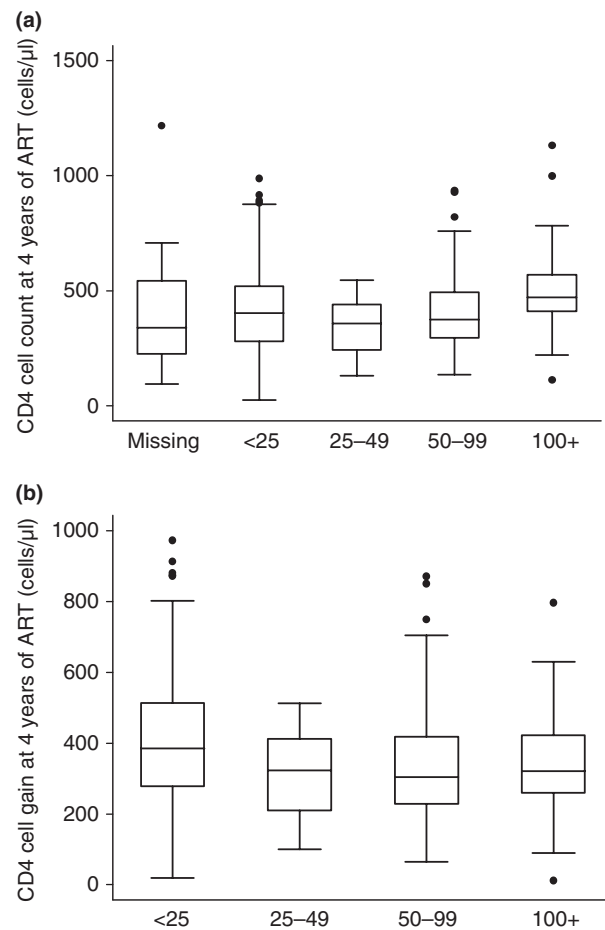


Figure 2 Immunological response at 4 years of therapy use, Phnom Penh, Cambodia. (a) Distribution of CD4 cell count measurements by initial CD4 cell value. (b) Distribution of CD4 cell gains by initial CD4 cell value. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles); whiskers' ends represent 5th and 95th percentiles, and dark dots outlier values.

severe high liver enzymes was taking NVP-containing therapy.

Virological failure and drug-resistant viruses

Fifteen individuals (4%) had detectable HIV-1 RNA, and all of these had a viral load of >1000 copies/ml (median 4.9 log₁₀; IQR = 3.7–5.3) 4 years after cART start. Three were receiving second-line therapy for a median of 20 months. Only one patient with virological failure had CD4 cell count <100 , and eight had <200 cells/ μ l. In an intention-to-treat analysis, 26.1% (95% CI 22.0–30.5) of cohort members had failed first-line cART after 4 years of use (114/437).

Genotyping reactions for two patients with detectable viral load (one on first-line and one on second-line treatment) failed. The CRF01AE HIV-1 subtype was isolated in all specimens genotyped. One patient treated with first-line and one with second-line therapy had wild-type viruses (Table 2). The most frequently observed mutations were M184V and K103N (nine and five patients, respectively). Five patients had thymidine analogue mutations (TAMs): one had TAMs of types 1 and 2; three TAMs of type 1 only, and one TAMs of type 2 only. Eleven individuals had mutations conferring ARV resistance to NRTIs and/or NNRTIs (Figure 3). Eight of these patients were ARV naïve at cART start, and 10 were receiving a drug related to the observed drug resistance at the time of the survey. Nine individuals had resistance to two drug classes (NRTI and NNRTI). Only one of the patients who were receiving second-line therapy had developed mutations but not resistance to protease after 5.7 months of second-line use. The overall frequency of cART resistance in the cohort was 2.6% (9/347).

Treatment adherence

After 4 years of cART, 83.3% of patients were considered fully adherent to cART, and 3.4% poorly adherent (Table 1). Sixteen per cent of patients with virological suppression reported suboptimal adherence compared to 40.0% of patients with detectable viral load (Fisher's exact $P = 0.03$). Mean difference in reported adherence between patients with detectable and undetectable viral load was 0.4% (Wilcoxon rank-sum $P = 0.01$). The two individuals with wild-type virus and four of the 11 patients identified with resistance reported poor adherence to cART. No difference in adherence was reported by men and women (Wilcoxon rank-sum $P = 0.17$).

Discussion

In this cohort of adults who started cART at an advanced stage of disease in Phnom Penh, 80% of patients were alive and receiving care after 4 years of therapy. Most patients were asymptomatic, received first-line regimens, were compliant with treatment and experienced sustained CD4 cell response to therapy. Only 4% of patients had detectable HIV viral load, and 10% were at the risk of developing opportunistic infections (CD4 cell count <200 cells/ μ l).

Despite late presentation of patients at programme entry and cART start (68% had CD4 cell count levels <50 cells/ μ l), after 4 years of therapy, 80% of patients

Table 2 Frequency of HIV-1 reverse transcriptase mutations at 4 years of antiretroviral therapy use among patients with HIV-1 RNA >1000 copies/ml, Phnom Penh, Cambodia

Patient	Drug regimens used since cART start	Mutations		Resistance		
		NNRTI	NRTI	NNRTI	NRTI	Protease
First-line therapy						
1	AZT-3TC-EFV, AZT-TDF-LPVr*	–	F116FY, Q151LM	No	Yes	NA
2	d4T-3TC-NVP	Y181C	D67N, K70KR, M184V	Yes	Yes	NA
3	d4T-3TC-EFV, AZT-3TC-NVP	Y181C	T69N, M184V, T215Y	Yes	Yes	NA
4	d4T-3TC-EFV, AZT-3TC-EFV, ddI-AZT-EFV	–	–	No	No	NA
5	d4T-3TC-EFV, AZT-3TC-EFV	K103N, P225H	M184V	Yes	Yes	NA
6	d4T-3TC-EFV, AZT-3TC-NVP	K103N, Y181C	M184V	Yes	Yes	NA
7	AZT-3TC-EFV, AZT-3TC-NVP	K101E, G190A	M184V, T215F	Yes	Yes	NA
8	AZT-3TC-EFV*	K103N, M230L	M41L, M184V, D67N, T69D, L74V, M184V, L210W, T215Y	Yes	Yes	NA
9	d4T-3TC-EFV*	L100I, K103N	K70R, M184V, T215I, K219E	Yes	Yes	NA
10	d4T-3TC-EFV	K103N	K65R M184V	Yes	Yes	NA
11	AZT-3TC-NVP	K103KN	M184V	Yes	Yes	NA
Second-line therapy†						
12	d4T-3TC-EFV, AZT-3TC-NVP, ddI-ABC-LPVr	Y181C	–	Yes	No	No
13	d4T-3TC-EFV, AZT-3TC-LPVr	–	–	No	No	No

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; cART, combined antiretroviral therapy; d4T, stavudine; ddI, didanosine; EFV, efavirenz; LPVr, boosted lopinavir; NA, not applicable; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine.

*Antiretroviral experienced at therapy start.

†Patient number 12 had no protease mutations. Patient number 13 had the following mutations not conferring resistance to protease inhibitor drugs: H69K, I13V, L10I, M36I, V82I.

remained in care, and most of them continued experiencing CD4 cell count gains similar to or higher than those described in the literature (Garcia *et al.* 2004), even in studies conducted among patients who maintained viral load suppression for several years (Mocroft *et al.* 2007; Kelley *et al.* 2009). Furthermore, almost one-third of the patients had reached 500 CD4 cells/ μ l at 4 years of treatment, and greater CD4 count increases were observed in patients with lower initial CD4 counts. These findings are also consistent with previous studies (Yamashita *et al.* 2001; Garcia *et al.* 2004; Egger *et al.* 2009; Kelley *et al.* 2009). Given that both advanced clinical

disease and low pre-therapy CD4 count levels have been identified as important determinants of poor immunological response and maximum CD4 values achieved on therapy (Hunt *et al.* 2003; Etard *et al.* 2006; Mocroft *et al.* 2007; Bussmann *et al.* 2008; Nash *et al.* 2008), earlier start of treatment could further improve long-term patient outcomes in our programme. Besides the observed favourable immunological outcomes, which are consistent with sustained viral load responses over time (Mocroft *et al.* 2007; Egger *et al.* 2009), only 4% of the patients who remained in care after 4 years of cART (or 26% of the cohort in the intention-to-treat analysis) failed to

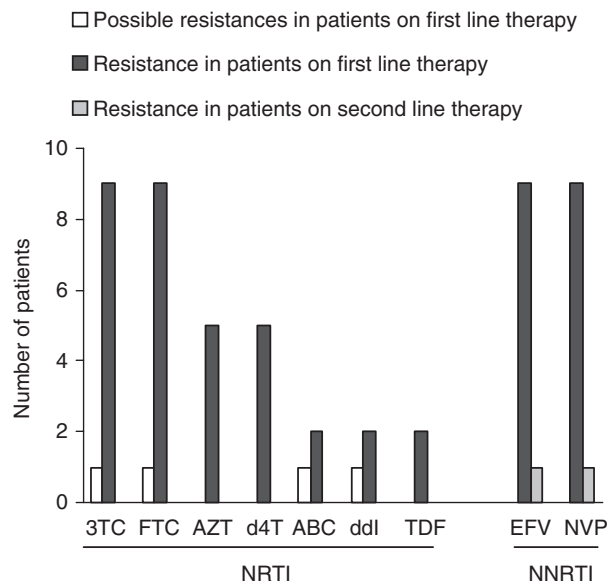


Figure 3 Frequency of HIV antiretroviral resistance by drug class and treatment line at 4 years of therapy use, Phnom Penh, Cambodia. 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; FTC, emtricitabine; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; TDF, tenofovir.

suppress HIV. These results are similar to or better than the findings from previous evaluations conducted in Gaborone, Botswana and Dakar, Senegal (Etard *et al.* 2006; Bussmann *et al.* 2008). In the study from Gaborone, Kaplan–Meier estimates of death or virological failure of 22% at 3 years ($n = 327$) and 30% at 5 years ($n = 133$) were reported. In Senegal, 44% of 97 patients had unsuppressed viral load after 4 years of cART use. We did not observe differences in immunovirological responses in men and women treated in our cohort. This lack of association has also been reported by a number of previous studies (Srasuebkul *et al.* 2007; Egger *et al.* 2009). However, other researchers found greater immunological recovery in women than in men (Gandhi *et al.* 2006; Collini *et al.* 2009). Discrepancies across studies might be explained by sex-related differences in initial clinico-immunological characteristics and/or in adherence levels.

It is important to acknowledge that mortality and loss to follow-up rates before the survey might have led to overestimate virological suppression and immunological recovery rates, as some of the deaths or dropouts (especially those occurring after the first 6 months of therapy) could primarily represent non-compliant patients

and poor respondents to therapy. However, the intention-to-treat analysis also showed good results with an overall cumulative failure rate of first-line therapy of 26% at 4 years of treatment. Furthermore, participation rates for the clinical and laboratory components of the study were very high (98% of eligible patients) minimizing the possibility of participation biases.

The CRF01_AE strain, responsible for over 95% of infections in Thailand, Cambodia and Vietnam (Hemelaar *et al.* 2006), was isolated in all specimens genotyped. Of the 13 patients with virological failure and genotyping data, nine had mutations to two drug classes, and 11 had drug resistance. The most prevalent resistance patterns were against NRTIs (3TC/FTC or AZT/d4T) and/or the two NNRTIs prescribed (EFV/NVP). This is consistent with reports of associations between NNRTI-based therapy and the development of resistance to multiple drug classes, probably because of the susceptibility to select for resistant virus to both 3TC and NNRTI (Harrigan *et al.* 2005; Tam *et al.* 2008).

The good immunovirological outcomes observed in our cohort are consistent with the majority of our patients achieving sustained levels of adherence to therapy over time. In our programme, FDC combinations of generic ARV drugs were given to the patients, and a dedicated team of experienced non-medical counsellors provided psychosocial and adherence support at the time of clinical visits. In the study, we assessed self-reported patient compliance with cART over the past month using the VAS tool. Despite the high thresholds of adherence used to classify patient adherence status (100%), more than 80% of patients were considered compliant with treatment regardless of the method used. The observed similar adherence levels reported by men and women has also been reported by a recent study in Botswana (Bussmann *et al.* 2008).

Toxicity attributed to ARV use was frequently diagnosed in our patients, especially dyslipidemia and d4T-related toxicity. Similar findings have been reported by researchers in Thailand and Singapore (Paton *et al.* 2002; Homsanit *et al.* 2007). Dyslipidemia diagnosed in HIV-infected patients has been associated with an increased risk of myocardial infarction of 1.2 per year of cART use in European cohorts (Friis-Møller *et al.* 2003). Our results suggest that counselling to reduce alcohol consumption, regular assessment of patients' cardiovascular risk and use of non-pharmacological approaches, such as diet and exercise, also need to be considered in the long-term management of patients receiving cART in resource-limited settings. In our cohort of patients primarily treated with d4T-based first-line therapy, lipodystrophy was diagnosed in 63% of patients (severe symptoms in 7%),

and peripheral neuropathy in 25%. Prevalence and severity of mitochondrial toxicity are known to increase with the duration of treatment (Carr & Cooper 2000), and several studies have reported strong associations between presence of lipodystrophy and d4T use (Hurst & Noble 1999; Saint-Marc *et al.* 1999; Carr & Cooper 2000; Fellay *et al.* 2001). This condition has also been observed more frequently in patients with low nadir CD4 count (Mauss *et al.* 2002). In clinical practice, physicians might tend to underestimate the frequency and anxiety associated with the presence of minor or mild adverse events (Justice *et al.* 1999). However, real or anticipated drug side effects have been identified as important barriers to treatment adherence in several studies (Basseti *et al.* 1999; Ammassari *et al.* 2001, 2002; Weiser *et al.* 2003), and non-life threatening toxicity, even if of minor severity, could lead to suboptimal adherence to therapy, treatment discontinuation and failure. As recommended in new WHO guidelines (World Health Organization, 2010), the use of better tolerated first-line ARV regimens containing TDF or AZT would help decreasing the development of long-term toxicity in patients receiving cART. Supportive open discussion and education of patients to balance benefits and constraints related to the use of therapy are simple key strategies that can help to ensure long-term adherence to cART (Mills *et al.* 2006).

In conclusion, the results of this study support the feasibility and long-term effectiveness of the care strategy adopted by the MSF-supported HIV/AIDS programme in Phnom Penh, which employed a combination of regular clinical follow-up visits by physicians, regular routine CD4 cell count monitoring, use of cART FDC and the provision of regular adherence and psychosocial support led by well-trained counsellors.

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Corresponding Author Mar Pujades-Rodríguez, MSF/Epicentre; 8, rue St. Sabin, 75011 Paris, France. Tel.: +33 4 7211 5736; Fax: +33 4 7211 5141; E-mail: mar.pujades@epicentre.msf.org